Priority

Applicants respectfully traverse the Examiner's statement that the claims are not entitled to priority of U.S.S.N. 09/496,914. The sequence recited in claim 32 is 91% identical to SEQ ID NO: 4498 disclosed in U.S.S.N. 09/496,914; thus, at least claim 32, in addition to other claims reciting corresponding sequences, are entitled to priority of that application's filing date of February 3, 2000.

Drawings

The Examiner has objected to the drawings because the number designations for each figure describing SEQ ID NO:4 are allegedly not consistent with the specification and sequence listing. Applicants have corrected the drawing and submit herewith corrected drawings. The original drawing showed a Blastx alignment of SEQ ID NO:2 (nucleotide) with mouse C-type lectin receptor amino acid sequence and was mislabeled as a Blastp alignment. The drawing has been corrected by performing a Blastp alignment comparing SEQ ID NO:4 (amino acid) to the mouse C-type amino acid sequence.

Examiner's Position

In the Office Action dated February 27, 2002, the Examiner made the following rejections:

(1) Claims 10-11, 20, and 30-31 were rejected under 35 U.S.C. §101 as allegedly not supported by a specific, substantial and credible utility, or a well-established utility;

(2) Claims 10-11, 20, and 30-31 were rejected under 35 U.S.C. §112, first paragraph, for asserted lack of enablement; and

(3) Claims 10-11, 20, and 30-31 were rejected under 35 U.S.C. §112, first paragraph, for asserted lack of written description of the genus of polypeptides comprising the sequence of SEQ ID NOS:4 or 6, or sequences 99% identical thereto.

(4) Claims 31 was rejected under 35 U.S.C. §112, second paragraph, as being indefinite for the lack of sufficient antecedent basis for the recitation of "the polynucleotide".

Applicants traverse each of these rejections as follows.

35 U.S.C. §101 Utility Rejection Should Be Withdrawn

The rejection under 35 U.S.C. § 101 should be withdrawn because the specification does teach a specific, substantial, and credible utility for the claimed polypeptides.

I. SEQ ID NO:4 is a member of the C-type lectin receptor family.

The specification teaches a C-type lectin receptor-like polypeptide of SEQ ID NO: 4, that is an approximately 234 amino acid protein with a predicted molecular mass of approximately 26kDa unglycosylated. A predicted twenty residue transmembrane region is encoded from residue 24 to residue 36 of SEQ ID NO:4. A predicted extracellular portion is encoded beginning at approximately residue 42 of SEQ ID NO:4. The extracellular portion (also represented as SEQ ID NO:6) is useful on its own as a soluble protein. A predicted N-linked glycosylation site is encoded between residues 110 and 112 (Arg His Trp) of SEQ ID NO:4, which can be confirmed by expression in mammalian cells and sequencing of the cleaved product (See Specification, page 4, lines 25-31 to page 5, lines 1-2).

a. Homology with other C-type lectin Family Members

As disclosed in the specification at page 4, lines 5-24, the claimed polypeptide is homologous to members of the C-type lectin receptor family. SEQ ID NO:4 exhibits homology with mouse macrophage C-type lectin receptor, human dendritic cell immunoreceptor (DCIR), human C-type lectin receptor DDB27, and mouse C-type as shown in Figures 1 to 4 of the specification and attached hereto as Exhibit 1. The polypeptide of SEQ ID NO:4 is 39% identical to mouse macrophage C-type lectin, 49% identical to human dendritic cell immunoreceptor (DCIR), 49% identical to human C-type lectin receptor DDB27, and 44% identical to mouse C-type. In addition, SEQ ID NO:4 exhibits 99% identity the blood dendritic cell antigen 2 (BDCA-2), a plasmacytoid dendritic cell-specific Type II C-type lectin (Dzionek, *et al., J. Exp. Med.* 194(12):1823-1834 (2001); International Patent Application No. WO 01/36487 A2) (Exhibit 2). This homology to the known C-type lectin receptor family members strongly suggests that C-type lectin receptor-type polypeptide of the present invention is a novel

member of the C-type lectin receptor family and would possess similar biological activity as the known C-type lectin family members. One of ordinary skill in the art accepts homology based on amino acid sequence identity as a credible method of determining the function of a polypeptide. *See* Henikoff, *et al., Science*, 278:609-614 (1997).

b. Essential domains of C-type lectin receptor proteins are conserved.

An alignment of SEQ ID NO:4 with the amino acid sequences of other C-type lectin receptor proteins, including BDCA2/dendritic cell lectin (DLEC), DCIR, C-type lectin 6 (CLEC-6) is shown in Exhibit 2. The C-lectin domain extends from amino acid 100 to amino acid 206 of SEQ ID NO:4, and the C-type consensus sequence extends from amino acid 180 to 206, as determined by PROSITE analysis (Falquet, *et al., Nucl. Acid Res.* 30:235-238 (2002)). A mannose or glucose binding specificity is derived from the amino acid motif, glutamic acid/proline/asparagines (EPN), starting from amino acid 172 to 174 of SEQ ID NO:4 and DLEC/BDCA-2, similar to mouse macrophage receptor. By comparison, DCIR has the variant amino acid motif, glutamic acid/proline/serine (EPS) which confers binding specificity for galactose, whereas hepatic asialoglycoprotein receptors 1 and 2 (ASGPR-1 and –2) have the amino acid motif, glutamine/proline/aspartic acid (QPD), that binds N-acetylgalactosamine. Type II members of the C-type lectin receptor proteins are characterized by a single carbohydrate recognition site (CRD) which has one calcium-binding domain per CRD, wherein the domain consists of calcium binding residues at amino acid 146, amino acids 172-174, 178-179 and amino acids 194-195. Thus, the polypeptide of SEQ ID NO:4 shares in common, the C-lectin domain, C-type consensus sequence, amino acid motifs specific for mannose or glucose binding and CRD with requisite calcium-binding domains and N-glycosylation sites, with other members of the C-type lectin protein family, particularly BDCA-2. The cysteines that participate in the disulphide bond formation and can potentially aid in dimerization are also conserved, four within the CRD domain at amino acid residues 111, 180, 198, and 206. There are two additional cysteines present at amino acid residues 82 and 94 that may be optionally involved in disulphide linkage. Accordingly, based on the high homology that SEQ ID NO: 4 shares with other members of this family and conservation of the C-lectin domain, C-type consensus sequence, amino acid motifs specific for mannose or glucose binding, CRD with requisite calcium-binding domains and N-glycosylation sites, and cysteines that participate in disulphide bond formation

and can potentially aid in dimerization, SEQ ID NO:4 has utility as C-type lectin receptor protein as asserted in the specification.

c. eMATRIX and Pfam Analyses are consistent with C-type lectin receptor polypeptide.

Using eMATRIX software package (Stanford University, Stanford CA) (Wu, *et al., J. Comp. Biol.* 6:219-235 (1999)), the C-type lectin receptor-like polypeptide of SEQ ID NO: 4 was determined to have the following eMATRIX domain hits. The results describe: e value, source, Accession number, domain name, amino acids of the full-length protein encoded by the polynucleotide of SEQ ID NO:4 that correspond to the eMATRIX domain and nucleotides of the open reading frame of the SEQ ID NO:4 that correspond to the domain: (1) 2.731e-09, 12.25, BL00615B, C-type lectin domain proteins, amino acids 193-206, WNDIHCHVPHKSIC and (2) 9.400e-09, 16.68, BL00615A, C-type lectin domain proteins, amino acids 94-111, CYFISTGMQSWTKSQKNC. Thus, the eMATRIX results describe the presence of C-type lectin domain proteins consistent with SEQ ID NO: 4 having C-type lectin receptor-like activity and being a member of the C-type lectin receptor family.

Using the Pfam software program (Sonnhammer, *et al., Nucleic Acids Res.* 26:320-322 (1998)), C-type lectin receptor-like protein of SEQ ID NO:4 is predicted to contain one (1) lectin C-type domain wherein the score is 97.7, E-value 2.3e-25, and amino acid sequence encoded (start and end amino acid position) is: GMQSWTKSQKNCSVMGADLVVINTTEEHDFIIHNL KRNSSYFLGLSHPRGRRHWQWVDHTPYNENvTFWHSGEPNNLDERCAIINFRSSQEWG WNDIHCHVPHKSICEM (100-208).

In summary, overall homology with known members of the C-type lectin receptors, conservation of the active sites, and eMATRIX and Pfam results are consistent with the assertion that SEQ ID NO: 4 is a member of the C-type lectin receptor family.

II. SEQ ID NO:4 has specific, substantial and credible utility as a C-type lectin receptor-like polypeptide.

Members of the C-type lectin receptor serve as antigen receptors and regulate the migration of dendritic cells and their interaction with lymphocytes (Figdor, *et al., Nature*

Reviews – Immunology 2:77-84 (2002)), and can be used, *inter alia*, in a screen for immune-related disorders and the management and/or treatment of immune-related disorders (Hazku *et al., Am J. Respir. Crit. Care Med.* 161:952-960 (2000); Kleinau *et al., J Immunol* 162(7):4266-4270 (1999); Mossalyi *et al., Blood.* (1990) 75:1924-1927; Ouaaz *et al., Blood.* (1994) 84(9):3095-3104; Fournier *et al., Blood.* (1994) 84(6):1881-1886). For example, plasmacytoid dendritic cell-specific antigen-2 (BDCA-2), a Type II C-type lectin, is known to mediate antigen capture and to modulate interferon α/β induction by viruses and inflammatory factors (Dzionek, *et al., J. Exp. Med.*194(12):1823-1834 (2001)). Thus, anti-BDCA-2 mAb may be useful in treating systemic lupus erythromatosus (SLE), an inflammatory, autoimmune disorder characterized by increased levels of IFN α/β. This utility is specific, inasmuch as it is not a utility shared by all polypeptides. This utility is also a real-world use because members of the C-type lectin receptor family are useful for screening immune-related disorders. In addition, therapies utilizing antibodies to such polypeptides could be developed to correct or ameliorate defects in the processes mediated by antigen recognition and immune cell interactions. The Specification teaches the use of these C-type lectin receptor polypeptides for the management and/or treatment of inflammatory disorders (See Specification, 5.8.7 IMMUNE STIMULATING OR SUPPRESSING ACTIVITY, page 55, line 6 to page 59, line 17; 5.8.15 ANTI-INFLAMMATORY ACTIVITY, page 68, line 12 to page 69, line 4; 5.8.18 ARTHRITIS AND INFLAMMATION, page 71, line 26 to page 72, line 11)

a. mRNA expression of SEQ ID NO:4 supports a role in immune-related reactions

As described in the Declaration of Walter Funk, Ph.D., PCR studies demonstrated in Table 1 (Exhibit 3) that SEQ ID NO:4 mRNA was expressed almost exclusively in immune cells and tissues. The expression of SEQ ID NO:4 was observed in resting CD4+ cells and CD19+ cells, but not activated CD4+ cells and activated CD19+ cells. The findings that C-type lectin receptor-like expression is detected in resting B-cells and T-cells but not in activated B-cells and T-cells suggest a role for this protein in lymphocyte activation and/or differentiation similar to that of another type II C-type lectin receptor, the CD23 (Mossalyi *et al., Blood.* (1990) 75:1924-1927; Ouaaz *et al., Blood.* (1994) 84(9):3095-3104; Fournier *et al., Blood.* (1994) 84(6):1881-1886). Similarly, DCIR to which SEQ ID NO:4 has significant homology, was shown to be

expressed on myeloid CD14+ cells and DCIR expression is down-regulated upon cell maturation (Figdor, *et al., Nature Reviews – Immunology* 2:77-84 (2002)).

In addition, expression of SEQ ID NO:4 mRNA was detected in peripheral blood mononuclear cells, tonsil, spleen, peripheral blood leukocytes, fetal spleen, placenta, lung and testis. As described in the specification, at page 43, lines 6-10, C-type lectin receptors are involved in inflammatory diseases, such as asthma. For example, C-type lectin receptor may play a role in modulating the inflammatory response associated with allergic airway disease by phagocytosis and antigen uptake (Currie, *et al., J. Immunol.* (2000), 164(7):3878-86). As mentioned above, CD23, a member of the same class of C-type lectin receptors as SEQ ID NO:4, also plays an important role in allergy and inflammation (Hazku *et al., Am J. Respir. Crit. Care Med.* 161:952-960 (2000); Kleinau *et al., J. Immunol.* 162(7):4266-4270 (1999)). These findings that C-type lectin receptor-like expression is detected in immune cells and tissues are consistent with a role of C-type lectin receptor-like polypeptides in immune-related reactions, such as inflammation and asthma.

b. Western Blot analysis support assertion that SEQ ID NO:4 is a shed receptor similar to L-selectin, another C-type lectin receptor with immune-related activity

The specification teaches that as a predicted transmembrane protein, SEQ ID NO:4 may function as a shed receptor. In addition, the extracellular portion of SEQ ID NO:3 may be used as a biopharmaceutical (page 4, lines 27-31 to page 5, lines 1-2). The Examiner has noted on page 9 of the Office Action that "the specification asserts that SEQ ID NO:4 may function as a shed receptor, however the specification has not demonstrated such..." As described in the Declaration of Walter Funk, Ph.D. and Exhibit 4, Western Blot studies demonstrated that the polypeptide of SEQ ID NO:4 is secreted or cleaved from the cell surface.

The Examiner asserts that "The fact that a receptor may be shed does not make clear or apparent the function or specificity of the receptor, nor does it identify the ligand for the receptor." Applicants respectfully traverse. Another member of the C-type lectins, L-selectin, was shown to be shed as a result of activation of leukocytes (Tedder, *et al., FASEB J.* 9, 866-873 (1995)). L-selectin is a calcium-dependent C-type lectin known to mediate the rolling and tethering of leukocytes on endothelial surfaces, which is a prerequisite for leukocyte adhesion

and extravasation. In particular L-selectin mediates homing of naïve lymphocytes via endothelial veins to peripheral lymph nodes and Peyer's patches and also plays a role in recruitment of leukocytes to inflammatory sites. *In vitro*, association of L-selectin with GlyCAM-1 can activate beta2 integrins. L-selectin is expressed by most hematopoietic cells at some stage of differentiation and its localization at the tips of the microvilli is required for optimal adhesion. CD23, another type II C-type lectin, is also shed and the soluble portion has immune activity (Mossalyi *et al. Blood.* (1990) 75:1924-1927). Similarly, as shown in Exhibit 4 and described in paragraph 6 of Dr. Funk's Declaration, SEQ ID NO:4 was shown to be secreted or cleaved from the cell surface much like CD23 and L-selectin that are known to be shed. These findings are consistent with C-type lectin activity of SEQ ID NO:4 similar to that of L-selectin and CD23, other C-type lectin receptor family members.

The Specification teaches that the C-type lectin receptor-like polypeptide of SEQ ID NO:4 is homologous to plasmacytoid dendritic cell-specific antigen-2 (BDCA-2), a Type II C-type lectin, known to mediate antigen capture and to inhibit interferon α/β induction (Dzionek, *et al., J. Exp. Med.*194(12):1823-1834 (2001)). Analysis of BDCA-2 mRNA expression by PCR showed that BDCA-2 is selectively expressed in plasmacytoid dendritic cells (PDCs). Similar to other C-type lectin receptors, BDCA-2 also was shown to exhibit antigen uptake as demonstrated by studies using Ag-mAb complexes, and induced a rapid and transient rise in intracellular calcium, consistent with a role in antigen capture and a calcium-mediated signal transduction pathway. Blocking of BDCA-2 with monoclonal antibodies inhibited the induction of IFN α/β induction by viruses and inflammatory factors. The activity of C-type lectin receptors, including BDCA-2, and homologous proteins, such as SEQ ID NO:4, to mediate antigen capture and modulate inflammatory mediators, specifically IFN α/β, is a specific utility, inasmuch as it is not a utility shared by all polypeptides. Because of the ability of C-type lectin proteins to regulate immune activity, C-type lectin receptor-like polypeptides, such as SEQ ID NO:4, may be useful in the management and/or treatment of immune-related disorders. For example, systemic lupus erythromatosus (SLE), an inflammatory disease, is characterized by increased levels of IFN α/β, which play a role in the pathogenic mechanism of SLE. The administration of anti-BDCA-2 mAb to SLE patients provides another therapy for inhibiting IFN α/β production by PDCs. The Specification teaches the use of these C-type lectin receptor polypeptides for the management

and/or treatment of inflammatory disorders (See Specification, 5.8.7 IMMUNE STIMULATING OR SUPPRESSING ACTIVITY, page 55, line 6 to page 59, line 17; 5.8.15 ANTI-INFLAMMATORY ACTIVITY, page 68, line 12 to page 69, line 4; 5.8.18 ARTHRITIS AND INFLAMMATION, page 71, line 26 to page 72, line 11)

In summary, it is evident based on the arguments and information presented that SEQ ID NO: 4 is a member of the C-type lectin family which includes blood dendritic cell antigen 2 (BDCA-2), mouse macrophage C-type lectin receptor, human dendritic cell immunoreceptor DCIR, human C-type lectin receptor DDB27, mouse C-type lectin receptor, and L-selectin, which have well-established utilities as antigen receptors and regulators of the migration of dendritic cells and their interaction with lymphocytes (Figdor, *et al., Nature Reviews – Immunology* 2:77-84 (2002)). Additionally, data in the attached Declaration of Walter Funk, Ph.D. demonstrates that the mRNA which encodes the claimed polypeptide is expressed uniquely in immune cells and tissues such as resting B-cells and T cells, tonsils and spleen but not activated B-cells and T-cells, suggests a role of this lectin in the immune response including inflammation and other immune-related disorders. In addition, the observation that this receptor can be shed from the cell surface suggest that shedding may regulate its activity similar to what has been shown for other C-type lectins such as CD23 and L-selectin. The C-type lectin receptor-like polypeptide of SEQ ID NO:4, therefore has a specific and substantial utility as a mediator of these immune processes involved in inflammation. Consistent with the teachings of the specification, the utilities known by those of ordinary skill in the art, and the data presented in the attached Declaration, Applicants respectfully submit that it is clear that the polypeptides of the present invention can be used as prognostic and diagnostic markers for the presence of immune-related disorders and diseased states associated with autoimmunity, inflammation and infection (See Specification, page 3, lines 13-20).

For the reasons discussed above, Applicants respectfully submit that the claimed invention has a utility that is credible, substantial, and specific to the C-type lectin receptor family of proteins. Applicants thus respectfully request withdrawal of the rejection under 35 U.S.C. §101.

35 U.S.C. §112, First Paragraph, Rejections Should Be Withdrawn

The Claims are Enabled Since the Claimed Sequences Have Utility

The rejection of claims 10-11, 20 and 30-31 under 35 U.S.C. § 112, first paragraph, for asserted lack of enablement because the invention lacked utility should be withdrawn for the reasons discussed above with respect to utility.

The Claims Satisfy the Written Description Requirement

The rejection of claims 10-11, 20 and 30-31 under 35 U.S.C. § 112, first paragraph, for asserted lack of written description should be withdrawn because there is ample description of a sufficient number of species to support claims directed to a genus of polypeptides comprising the sequence of SEQ ID NO: 4, or sequences 99% identical thereto.

The production of a number of species that are encompassed within the claimed genus is described throughout the specification . For example, the specification teaches at page 34, line 25 to page 36, line 29 modified proteins, fragments and derivatives of sequences of proteins, fusion proteins, including Ig fusions, etc. Met-1, Flag tag, His tag, etc. The specification also teaches that such modifications retain the desired activity of the protein (page 35, lines 3-4) or that fragments and derivatives would be expected to retain protein activity (page 35, lines 10-13).

Furthermore, Applicants have not relied solely on an analysis based solely on homology or membership in a broad family, but provide herewith data from PCR studies of mRNA expression and Western blot analysis to confirm the function or activity shared in common with other members of the C-type lectin receptor family, and more specifically the type II members, such as BDCA-2.

35 U.S.C. §112, Second Paragraph, Rejection Should Be Withdrawn

Applicants have amended Claim 31 to overcome the rejection under 35 U.S.C. §112, second paragraph, by reciting instead "a polynucleotide," as recommended by the Examiner.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance, and a Notice of Allowance is respectfully requested as soon as possible. If there are any questions regarding these amendments and remarks, or if further discussion would expedite allowance of the claims, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

Date: July 25, 2002

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Appendix A: marked up version showing the changes made in the specification

In the Claims:

Please cancel claims 16-18, amend claim 31, and add new claim 32, as follows:

31. (Amended) An isolated polypeptide encoded by [the]a polynucleotide comprising the sequence of SEQ ID NO:3.

32. (New) An isolated polypeptide comprising an amino acid sequence that is 90% identical to the amino acid sequence of SEQ ID:4 or SEQ ID NO:6.